Genes, inflammation, and age-related diseases

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Chapter 10

General discussion

Main findings

The general objective of this thesis was to investigate associations between genetic variants involved in inflammation and epigenetics and age-related diseases. First we analyzed genetic variation in genes related to inflammatory processes, since inflammation is known to play an important role in age-related diseases like cardiovascular disease, cognitive decline and cancer. Analysis of polymorphisms of the genes that are key for regulation of the immune response might clarify the patho-physiology of age-related diseases like atherosclerosis. Therefore, we analyzed the association between genetic variation in pro- and anti-inflammatory genes and cardiovascular disease (chapter 2 & 3), cognitive function (chapter 4 & 5), and cancer (chapter 6)

In chapter 2 we investigated the relation between the C804A polymorphism in the pro-inflammatory gene Lymphotoxin-alpha (LTA) also known as Tumor Necrosis Factor-beta (TNF-β) and coronary and cerebrovascular events. The C804A polymorphism causes an amino-acid change from threonine (T) to asparagine (N) at codon 26 (1). The variant protein 26N is associated with a two-fold increase in the induction of cell-adhesion molecules in vascular smooth muscle cells (1). These adhesion molecules are implicated in cardiovascular disease which might explain the association of the polymorphisms in the LTA gene and the increased risk for incident stroke (2;3). Our results indicate that carriers of the 804A allele have an increased risk for the primary study endpoint consisting of coronary events and clinical strokes, primarily in men. Furthermore, we found that the association between the C804A polymorphism and the primary endpoint in males was mainly attributable to incident strokes.

In chapter 3 we assessed the relation between four promoter polymorphisms in the interleukin-10 (IL-10) gene and vascular events at old age. Carriers of the -2849AA genetype within the IL-10 promoter region have an increased risk for vascular disease, coronary and cerebrovascular. In IL-10 knock-out mice the absence of IL-10 leads to an increased susceptibility of atherosclerosis (4). Furthermore, a study using an overexpressing transgenic mice model and IL-10 null mice showed a marked difference in lesion size between the groups, the IL-10 null mice having far more lesion

formation when compared to the overexpressing mice model (5). We found that two of the main IL-10 haplotypes showed significant associations with vascular diseases compared to the reference haplotype with no variants present. This provides evidence that not only pro-inflammatory processes contribute to atherosclerosis but that also anti-inflammatory cytokines are implicated in vascular disease. These findings support the hypothesis that genetic programming of the inflammatory response may be relevant to the pathogenesis of atherosclerosis.

The relation between genetic variation in inflammatory genes and cognitive function was investigated in chapter 4 and 5. In chapter 4, we assessed the association between four polymorphisms within the ICE gene and cognitive function in an elderly population, since genetic variation in the gene coding for ICE influences expression and function of IL-1β. We found that two variants in the ICE gene significantly lowered IL-1beta levels, and that carriers of these variants performed better on all cognitive function tests. This indicates that low levels of the pro-inflammatory IL-1beta might be protective for memory and learning deficits. These results are consistent with earlier findings on inhibition of ICE coinciding with lower IL-1beta levels in the hippocampus and improved memory (6). ICE inhibitors might therefore become a therapeutic target for subjects to prevent cognitive decline.

In chapter 5, we found that genetic variation in the promoter region of the IL-10 gene is associated with decreased cognitive function in individuals without clinical evidence of a cerebrovascular event. This provides evidence that genetic variation in the IL-10 gene is a good marker for risk prediction of cognitive function. If these findings are confirmed and adequately explained on the basis of independent studies, screening patients for the IL-10 promoter polymorphisms may contribute to a better risk stratification of patients at increased risk for cognitive decline and additional preventive therapy may be warranted.

We have assessed the association between circulating levels and innate production capacity of pro-inflammatory cytokines in whole blood samples and cancer incidence and mortality in chapter 6.

High levels of the circulating inflammatory markers were associated with an increased risk of cancer incidence and death from cancer. However, high innate pro-inflammatory cytokine production capacity was only associated with an increased risk of death from cancer during follow-up. This indicates that circulating markers of inflammation are increased in cancer patients, probably by autocrine production of the cancer cells themselves, and that therefore the relation between circulating inflammatory markers and incident cancer might be disturbed by reverse causality. However our main finding was that subjects with a pro-inflammatory trait, i.e. subjects with a high innate cytokine production level, had an increased risk of dying of the consequences of cancer when they had developed a tumor. Hence, it may be hypothesized that by administration of antibodies that bind pro-inflammatory cytokines, survival time of cancer patients might be extended.

Furthermore, we studied genetic variation in genes related to epigenetics, to give additional insights into mechanisms that underlie age-related diseases. Since, epigenetics is a fairly new concept in the relation with age-related diseases, we have provided first evidence that this process can indeed play an important role in the patho-physiology of age-related diseases.

In chapter 7, we investigated the association between genetic variation in the CREB Binding Protein (CBP) gene and cognitive function. Many experimental studies have investigated the role of CBP in memory formation and cognitive dysfunction in animals (7-10). $CBP^{+/-}$ mutant mice have normal short-term memory, but deficiencies in long-term memory, object recognition, and contextual memory tasks (11). Moreover, loss of one functional copy of the CBP gene in humans underlies all abnormalities in Rubinstein-Taybi Syndrome patients, including mental retardation (12;13). Therefore it is likely that polymorphisms in the CBP gene have an effect on cognitive function. We have demonstrated an association between two polymorphisms in the CBP gene and cognitive function in an elderly population. The variant alleles of the intron 4CT and intron 3AC polymorphisms were associated with better cognitive performance in all cognitive domains at baseline and in follow-up. Furthermore, the haplotype with the variant alleles of these two

polymorphisms also showed a protective effect on cognitive function in all cognitive domains. Future research is warranted to assess the functionality of these polymorphisms on the expression of CBP levels and how these polymorphisms affect the gene expression mediated by CBP. When confirmed, screening patients for the CBP polymorphisms may contribute to a better risk stratification of patients at risk for cognitive decline and may improve individual treatment.

In chapter 8, we investigated the impact of genetic variation in the PCAF-gene on all-cause mortality, and mortality due to vascular events and cancer in the PROSPER-study and validated our findings in the WOSCOPS and GENDER study. We showed in these three large prospective studies that the -2481C allele in the PCAF promoter is associated with a significant survival advantage in elderly patients while carriers were protected against clinical and angiographic restenosis after percutaneous coronary intervention (PCI). Functional analysis showed that the -2481 G/C polymorphism is located in a functional region of the PCAF promoter, and modulation of PCAF gene expression was detectable in an animal model of reactive stenosis. Our observations promote the concept that epigenetic processes are under genetic control and that, other than environment, genetic variation in genes encoding HATs may determine susceptibility to coronary heart disease outcomes and mortality.

In chapter 9, we used an innovative concept in genetic epidemiology. We assessed the association between plasma cholesterol levels and cancer risk, free from confounding and reverse causality, by a Mendelian randomization study design using the ApoE genotype. We found that subjects, when grouped by their baseline levels of cholesterol, had an increased cancer risk when the cholesterol levels were lower. This risk remained even after adjustment for potential confounders. However, when we categorized subjects according to their ApoE genotype, which also resulted in groups with significantly different cholesterol levels, no increased risk for cancer risk was observed between groups. These findings suggest that low levels of cholesterol are not causally related to an increased risk of cancer and that treatment with cholesterol lowering agents does not increase cancer risk.

Clinical implications

We have shown that subjects carrying genetic variants coding for a high pro-inflammatory profile or a low anti-inflammatory profile have an increased risk to develop several age-related diseases. In the process of atherosclerosis, inflammatory mechanisms operate largely through cytokine secretion and their activation can cause plaque rupture, thrombosis, and acute ischemic symptoms (14-18). Anti-inflammatory and immunosuppressive mechanisms inhibit atherosclerosis and may be attractive targets for disease prevention and/or treatment (19). They include amongst others anti-inflammatory cytokines, protective antibodies, and regulatory T cells, and may be induced by immunization. These therapies could also play an important role in delaying the process of cognitive decline in old age (20). For example, inhibition of ICE with ICE inhibitors have shown to reduce the IL-1β production in the brain (21), indicating a potential therapeutic role for ICE inhibitors in subjects with cognitive decline.

Moreover, we have shown that besides inflammatory processes, epigenetic mechanisms could also play a role in age-related diseases. Epigenetic modifications, like histone acetylation, accumulate with ageing (22). It can be envisioned that when this accumulation comes to a halt, the development of age-related diseases might be delayed (23). Histone deacetylases (HDAC) inhibitors have been successfully introduced in clinical trials as anti-tumour agents. Recent findings identified HDACs as possible targets for therapy in cardiovascular disease (24). The investigators found that HDAC inhibition could reduce the size of myocardial infarction by ~50% in mice.

A better understanding on the role of epigenetic processes in age-related diseases might provide novel opportunities to understand disease pathology. This would provide the necessary knowledge platform for design of alternative treatment strategies aimed at interfering in these epigenetic processes.

Genetic epidemiology can also contribute to establishing the causal nature of environmentally modifiable risk factors, through the application of Mendelian randomization approaches. By making use of this Mendelian randomization approach we found that low cholesterol levels were not

causally associated with an increase in cancer risk. Low cholesterol levels are probably a result of an increased uptake of the cancer cells themselves. Cancer cells need cholesterol for their growth and proliferation (25;26). A sudden drop in plasma cholesterol levels can therefore be a predictive factor of an underlying tumour. Hence, in clinical practice, more attention could be paid to sudden drops in cholesterol levels as a prediction of cancer.

Future research

Mendelian randomization is a fairly new concept in genetic epidemiology. It has already proven to be a valuable tool, since Mendelian randomization provides an alternative way of dealing with the problems of causal inference in observational studies. Inferring causality from observational data is problematic as it is not always clear which of two associated variables is the cause and which is the effect, or whether both are common effects of a third unobserved variable, a confounder. In some instances the problem of causality and confounding can be resolved with randomized clinical trials, however this is not possible for a whole range of exposures like toxins and physical activity. For these questions Mendelian randomization can provide a solution.

The Mendelian randomization approach has developed rapidly over the past 5 years, and proof of principle is now abundantly available. For example, the MTHFR C677T polymorphism, that determines homocysteine levels, shows a consistent causal relation with stroke as was also shown in observational studies (27). In the relation with CRP levels and metabolic syndrome, where several CRP polymorphisms were used as a proxy for CRP levels, no causal relation was found (28). Furthermore, genome wide association studies will provide us with new genetic variants as proxies for intermediate phenotypes for Mendelian Randomization studies. Therefore, Mendelian randomization should be applied more in future research (29).

Genetic epidemiology has merely been based on investigating associations with genetic variation in genes related to the investigated mechanisms. This is called a candidate gene approach, which we also used in all studies reported in this thesis. However, most age-related diseases are not the result

of only one disturbance in one pathway. Age-related diseases are complex diseases where many pathways and mechanisms could play a role in the patho-physiology. To determine all mechanisms involved in age-related diseases, the candidate-gene approach is not sufficient. Nowadays, the genetic technologies are much more advanced in a way that we can screen the whole genome. These genome wide associations studies (GWA) are used to discover more genetic variants in various, mostly unknown, pathways associated with complex age-related diseases. When these genetic variants are being discovered, we can make a better risk profile per individual, and eventually implement personalized therapy based on the individual genetic risk profile.

One example of a new genetic study is the PHASE project (PHArmacogenomic study of Statins in the Elderly at risk for cardiovascular disease), which started in January 2009. In this project we perform a genome-wide scan in all 5804 participants of the PROSPER study in order to discover the genetic variation responsible for variation in lowering low-density lipoprotein levels, variation in clinical outcome, like cardiovascular disease, cancer and cognitive decline, and for the occurrence of adverse effects. By increasing the knowledge of which genetic variation is responsible for the variation in drug response, we can develop personalized cholesterol lowering drug therapy based on an individual's genetic make-up to obtain optimal cardiovascular event reduction, minimal side effects, and major cost reduction for society.

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